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NEUROPEPTIDE-Y (NPY) INCREASES TOTAL BLOOD FLOW IN THE TAIL, AND REDUCES CUTANEOUS MICROVASCULAR BLOOD FLOW IN THE TAIL AND FOOT OF THE RAT

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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The purpose of this study was to describe the in vivo effects of neuropeptide-Y (NPY) on total blood flow (BF_T) and on microvascular skin blood flow (BF_{sk}) in appendages of a whole animal model. This study is important to understanding the medical relevance of NPY in cardiovascular physiology. The tail and foot of the rat were used as the model. BF_{sk} and skin temperature (T_{sk}) were measured on the foot pad and mid-way along the tail with a laser Doppler flowmeter and a 40-gauge thermocouple. BF_T was measured mid-way along the tail (distal to the laser Doppler flowmeter) via venous occlusion plethysmography. NPY (16, 32, 64 and 128 μg/kg), norepinephrine (NE) (25, 50, 100, and 400 μg/kg), or saline alone was administered i.v. in 300 μl volume. NE caused marked reductions in blood flow at all three sites. The duration of the contraction was correlated with the dose. NPY caused immediate marked decreases

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19. in BF_{sk} and significant increases in tail BF_T, tail blood volume, and tail T_{sk}. The magnitude and duration of the changes in BF were correlated to dose. These results indicate that some vessels in the tail are dilating in response to NPY. The most likely site of this vasodilation is the arteriovenous anastoma (AVA), which are shunts between arterioles and veins. When AVAs are open, the blood volume in the tissue increases, and the BF through the tissue increases because of the decrease in resistance, and capillary BF_{sk} often decreases as blood follows the path of least resistance. T_{sk} also increases when AVAs are opened.

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Experiments reported herein were conducted according to the principles set forth in the Guide for the Care and Use of Laboratory Animals, Institute of Laboratory Animal Resources, National Research Council, DHHS Publications (NIH) 86-23 (1985).

INTRODUCTION

Neuropeptide-Y (NPY) is a highly vasoactive substance that is released from the same sympathetic adrenergic nerve terminals as norepinephrine (NE) (1-6). In vivo studies in man (5) and other mammals have demonstrated marked reductions in blood flow in response to exogenous NPY (4,7,8). In vitro studies of isolated blood vessels have shown that NPY causes contraction in some tissues, e.g., the cerebral, skeletal muscle, and coronary, but not all vessels (3,5,9). The vascular constriction induced by NPY is slow in onset, but prolonged, compared to NE (10). NPY causes vasoconstriction by its effect on non-adrenergic vascular receptors (three NPY receptors are recognized). It also both potentiates the effect of other vasoconstrictors via its effect on adrenergic receptors and modulates vasodilatory effects (11) of acetylcholine, adenosine, and NE (6,7,12).

NPY has been implicated in cardiovascular disease (10,13,14). It is released during periods of elevated sympathetic activity and stimulation (10). Its pronounced and prolonged vasoconstrictive effects in some peripheral vascular beds may precipitate tissue ischemia (15). Because of these characteristics, NPY may play a role in some peripheral vascular disorders, particularly those associated with severe stress such as prolonged cold exposure. It is also reasonable to hypothesize that NPY may play a role in peripheral nerve and tissue injury associated with cold exposure. There is a clear need to characterize the in vivo effects of NPY on peripheral blood flow

in a whole animal model to establish its medical relevance to peripheral vascular disorders.

The purpose of the present study was to describe the in vivo effects of NPY on total blood flow (BF_T) and microvascular skin blood flow (BF_{sk}) in appendages of a whole animal model: the tail and foot of the rat (16). The effect of NPY was compared with that of saline (control) and of NE, a well-known vasoconstrictor.

METHODS

Animals. Eight male Long-Evans rats of 290-310 g were studied. At least one week prior to experiments, rats anesthetized with ~50 mg/kg sodium pentobarbital were equipped with a cannula (ITTC Life Science, S-26) in a jugular vein. Sterile surgical technique was used. In addition, hair was removed from the tail with a depilatory so it would not interfere with laser Doppler blood flow measurements.

Measurements. Air (T_a) and skin temperatures (T_{sk}) on the tail and foot were measured with thermocouples. Tail BF_T was measured by venous occlusion plethysmography (17,18) by use of mercury-in-silastic strain gauges (D.E. Hokanson Inc., 4.5 cm, wrapped twice around tail) connected to an electronic plethysmograph (19) (D.E. Hokanson Inc., model EC-4). BF_{sk} of the tail and foot was monitored with laser Doppler flowmeters (20,21) (TSI Laserflo, Model BPM 403A).

Preparation for experiments. Rats were anesthetized with ~50 mg/kg sodium pentobarbital and gently introduced into a

cylindrical plexiglas rat restrainer. The restrainer allowed free access to the hind leg and tail and to the end of the cannula located between the shoulders. A laser Doppler flow probe and a thermocouple were positioned on the skin adjacent to each other mid-way along the tail. A pneumatic cuff at the base of the tail and a mercury-in-silastic strain gauge ~2 cm distal to the laser Doppler probe were used to measure tail BF_T. A second laser Doppler flow probe was placed on the right foot, and a thermocouple was taped in the same position on the left foot.

All instrumentation was connected to a IBM compatible computer via an A/D converter (Keithley). Data collection was directed by LabTech Notebook software (Laboratory Technologies Corp.). Thermocouple and laser Doppler channels were sampled at 1-s intervals and averaged for 20 s. The venous occlusion plethysmographic measure of tail BF_T was done at 20-s intervals. The occlusion cuff was inflated to 55 mmHg for 5 s, and tail BF_T was assessed between the 2nd and 4th s of the cuff inflation.

Protocol. Fully instrumented rats rested in the restrainer for 15-30 min before the intravenous administration of NPY (16, 32 or 64 μg/kg), NE (25, 50, 100, 400 μg/kg), or saline control. The volume of all injections was 300 μl. Baseline recording was begun 5 min preceding the injection, and measurements were continued for at least 35 min post-injection. All experiments were done at T_a of 24-26°C.

RESULTS

NPY. Figures 1-3 illustrate the effect of NPY on tail BF_T and BF_{sk} in the foot pad and tail. Figs. 1 (absolute values) and 2 (as percent of baseline) show the effect of 64 μg/kg NPY on T_{sk} (Fig. 1a) and blood flow (Figs. 1b, 2a, 2b) in the tail and foot of a rat. The changes in all BF parameters can be described as having an immediate dynamic component followed by a prolonged static component. NPY caused an immediate increase in tail BF_T that normally peaked within 1-3 min. Tail volume (i.e., tail blood volume) also showed an immediate increase (>1.5%) and followed the same time course as tail BF_T. Tail T_{sk} also increased markedly (Fig. 1a), although it lagged the increase in tail BF_T slightly. In contrast, BF_{sk} in the tail and foot declined precipitously to <50% of baseline and bottomed-out within 1-3 min. There was no large or immediate change in foot T_{sk}.

After their dynamic increase, tail BF values (BF_{sk} and BF_T) began to return toward their respective baseline levels, albeit without reaching baseline during the 35 mins of post-injection data collection. Although tail BF_{sk} initially was a mirror image to tail BF_T, it typically declined again, completely independent of tail BF_T. Foot BF_{sk} did not return toward baseline, but continued at its new reduced level. BF values did not return to baseline even in experiments in which data were collected for an additional 40-60 min. These responses were characteristic for a

dose of 64 μg/kg (Fig. 3), although there were between rat variations in the magnitude of responses.

In a few rats, a dose of 128 μg/kg was used, but the effect on blood flow was not demonstrably different from that of 64 μg/kg. At the lower doses of 16 and 32 μg/kg, both the magnitude and the duration of the initial dynamic response was less. Furthermore, BF did return to baseline during the 35 min of post-injection observation (Fig. 3). As was expected, saline, administered i.v. as a control, had no significant effect on any of the parameters measured (Fig. 3).

Norepinephrine. In contrast to NPY, NE caused a reduction in both tail BF_T and in BF_{sk} in the tail and foot (Fig. 4a-c). For doses as large as 400 μg/kg NE, the reduction in blood flow was prolonged ~10 min, whereas the effect of smaller doses was of shorter duration (50 or 100 μg/kg: 1-3 mins, etc.). Tail blood volume was reduced in response to NE, a marked contrast to its increase in response to NPY.

DISCUSSION

The most important observations made in this study are (a) that NPY causes an increase in tail BF_T, not a decrease as was anticipated and (b) that NPY causes a decrease in BF_{sk} in the tail and foot. The observation that NPY increased BF_T in the rat tail was surprising considering the numerous reports that NPY causes either vasoconstriction or no change in vascular tone in blood vessels and reduced blood flow in organs and tissues heretofore studied (3,5,9). NPY has been reported to cause

constriction in cerebral, skeletal muscle, and coronary arteries, and iliac and femoral veins in the guinea pig and rat (see 12). The response is also striking in its contrast to the effect of NE (Fig. 1 vs Fig. 4).

The simultaneous increase in tail blood volume and BF_T indicates that some vessels in the tail are dilating in response to NPY. In vitro studies in the rat tail artery indicate that NPY causes a slow depolarization and concentration dependent contraction via a direct effect on smooth muscle cells (22). Our own observations show that microvascular flow was diminished after administration of NPY (Fig. 2b). This limits the possible sites of vasodilation to arterioles, venules, veins, and arteriovenous anastomose (AVAs). AVAs, which are shunts between arterioles and venules, are the likely candidates. They occur in large numbers in the rat tail and play a role in thermoregulatory heat loss. AVAs divert blood away from the capillaries as the blood follows the path of least resistance. The AVAs allow for a much higher rate of blood flow through a tissue than do the capillaries. When AVAs are open, the volume of blood in the tissue is greater because the functional vascular space is increased. The observed increase in tail T_{sk}, even while BF_{sk} was diminished (Fig. 1a), lends strong support to the suggestion that NPY increases tail BF_T by dilating AVAs.

The reduction in resistance that accompanies vessel dilation is arguably responsible for the increase in tail BF_T. The reduction in BF_{sk} may be due to either active vasoconstriction or

to the decrease in resistance in larger vessels causing blood flow to bypass the cutaneous capillaries. The observations that (a) tail BF_{sk} decreases when tail BF_T increases and (b) that tail BF_{sk} increases again just as BF_T begins to decline favor the latter explanation. There is another interesting observation about tail BF_{sk}. After initially being a mirror image of BF_T, it typically declines a second time (completely independent of BF_T) and remains low for the duration of the experiment. This could be due to either the effects of systemic or local changes in blood pressure or to a real constriction in this cutaneous vascular bed.

The foot BF_{sk} is distinct from that in the tail (Fig. 1b, 2b). Both the BF_{sk} and T_{sk} of the foot pad are higher than in the tail. While foot BF_{sk} declines immediately and remains quite low, foot T_{sk} remains nearly constant. It neither increases like tail T_{sk} nor decreases in response to the reduced blood flow. The lack of an increase in T_{sk} suggests that the vasodilation observed in tail BF_T does not occur in the leg or foot. Furthermore, no marked decrease in T_{sk} should be expected since the experiments are carried out in a thermally neutral environment where heat loss would be small.

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FIGURE LEGENDS

Figure 1. Effect of 64μg/kg NPY, administered i.v. to a rat, on tail and foot T_{sk} (1a) and on tail BF_T, and BF_{sk} in the tail and foot pad (1b). All data are expressed as absolute values.

Figure 2. BF_{sk} and tail BF_T in response to 64 μg/kg expressed as a percent of their mean baseline levels. Standardizing the BF data in this way allows for a reasonable comparison of the magnitude of changes at each site.

Figure 3. Dose response relationship of NPY on tail BF_T. The means for baseline and each consecutive 5-min period following the injection are shown to illustrate both the dynamic and longer-term static effects of NPY. Doses of 16, 32, and 64 μg/kg are compared with saline control. All injections were 300 μl in volume.

Figure 4. Effect of 400 μg/kg NE, administered i.v. to a rat, on total blood flow to the tail, and cutaneous microvascular flow in the tail and foot. Top (a) figure provides absolute values. Middle (b) and bottom (c) figures illustrate responses as percent change from baseline levels so that the magnitude of changes can be compared properly.

Tail & Foot Skin Temperatures
64 µg/kg Neuropeptide-Y

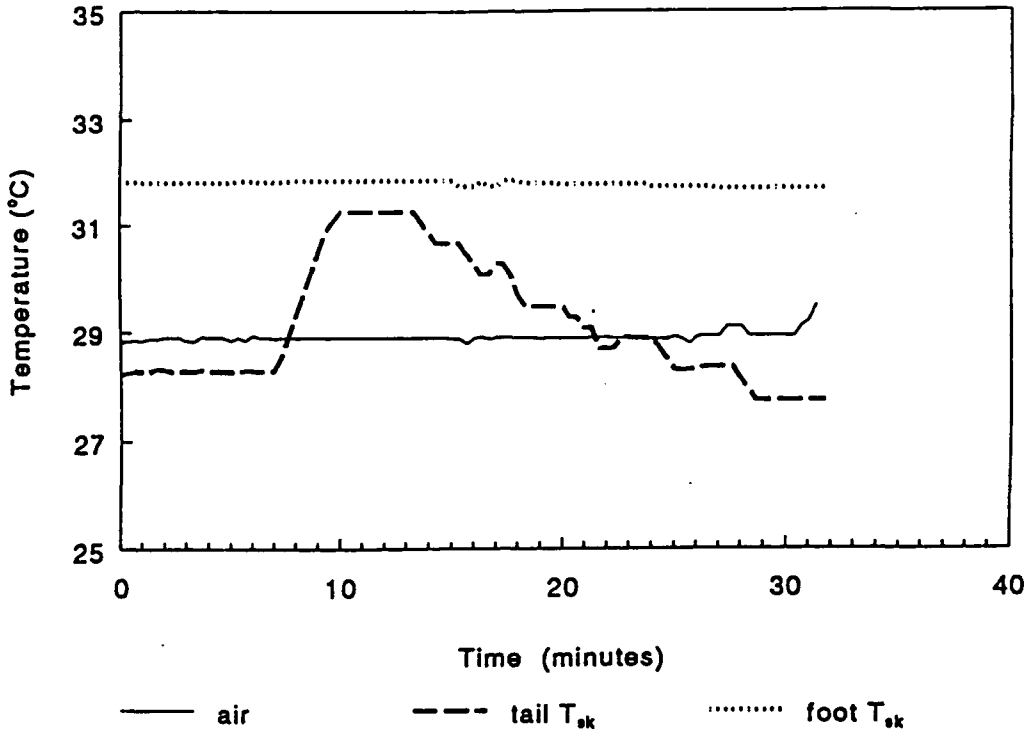


Figure 1a

Tail & Foot Blood Flow - absolute values
64 µg/kg Neuropeptide-Y

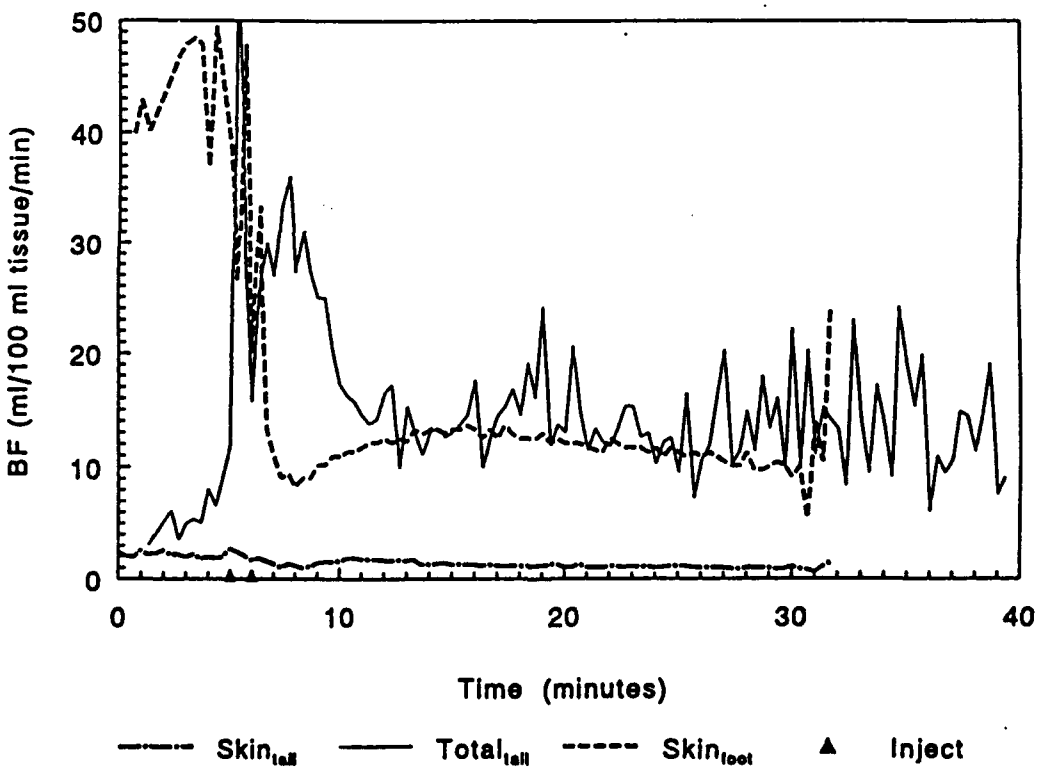


Figure 1b

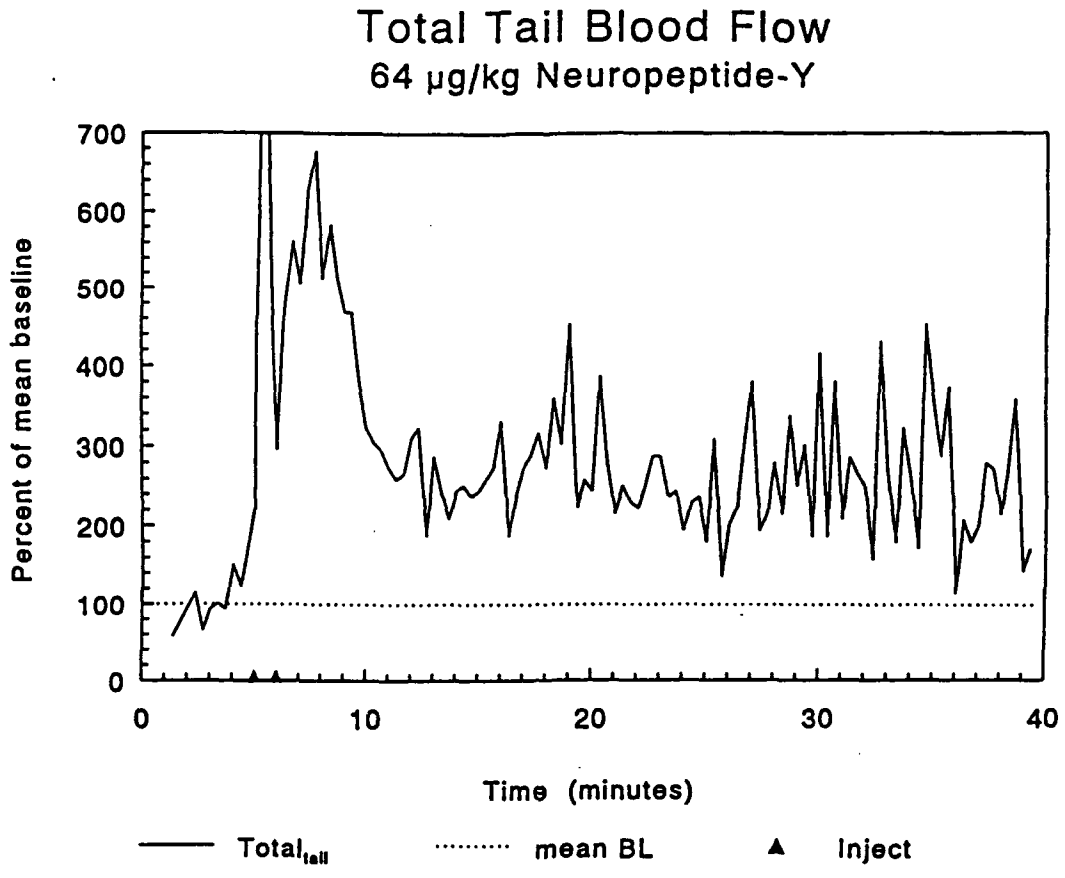


Figure 2a

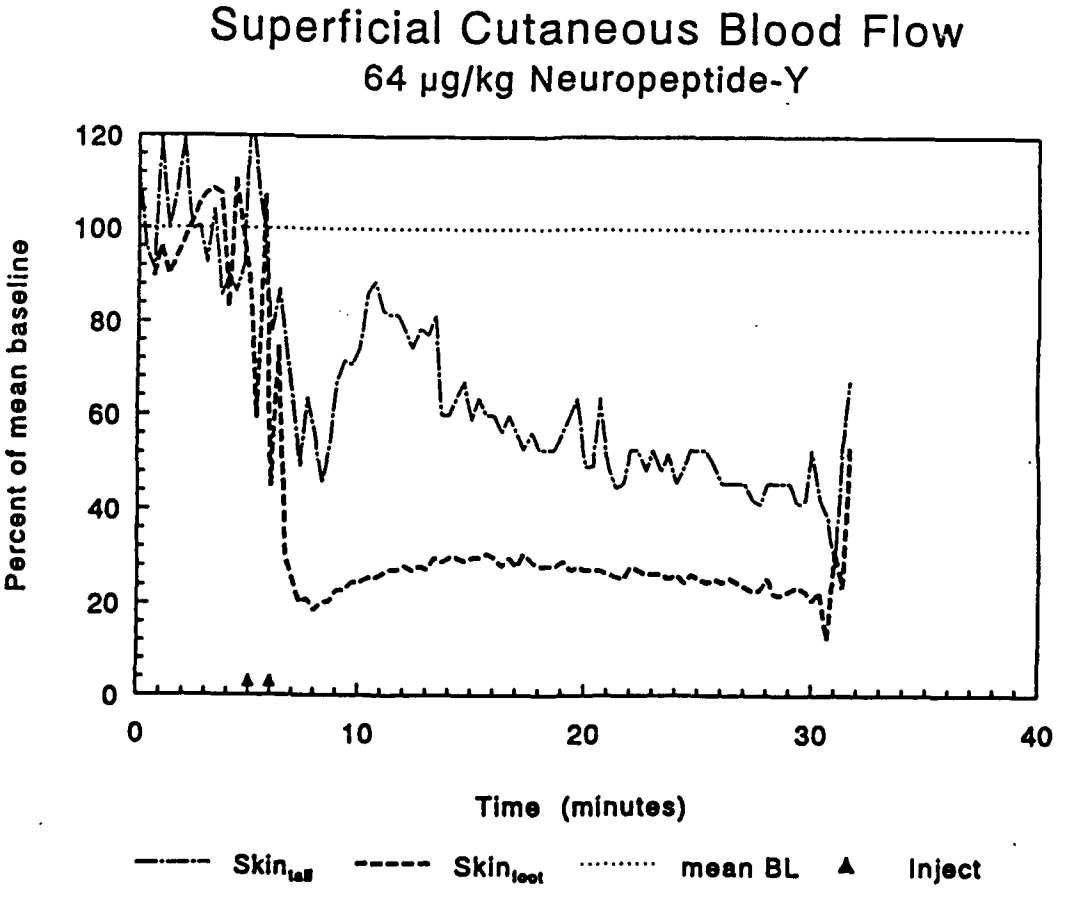
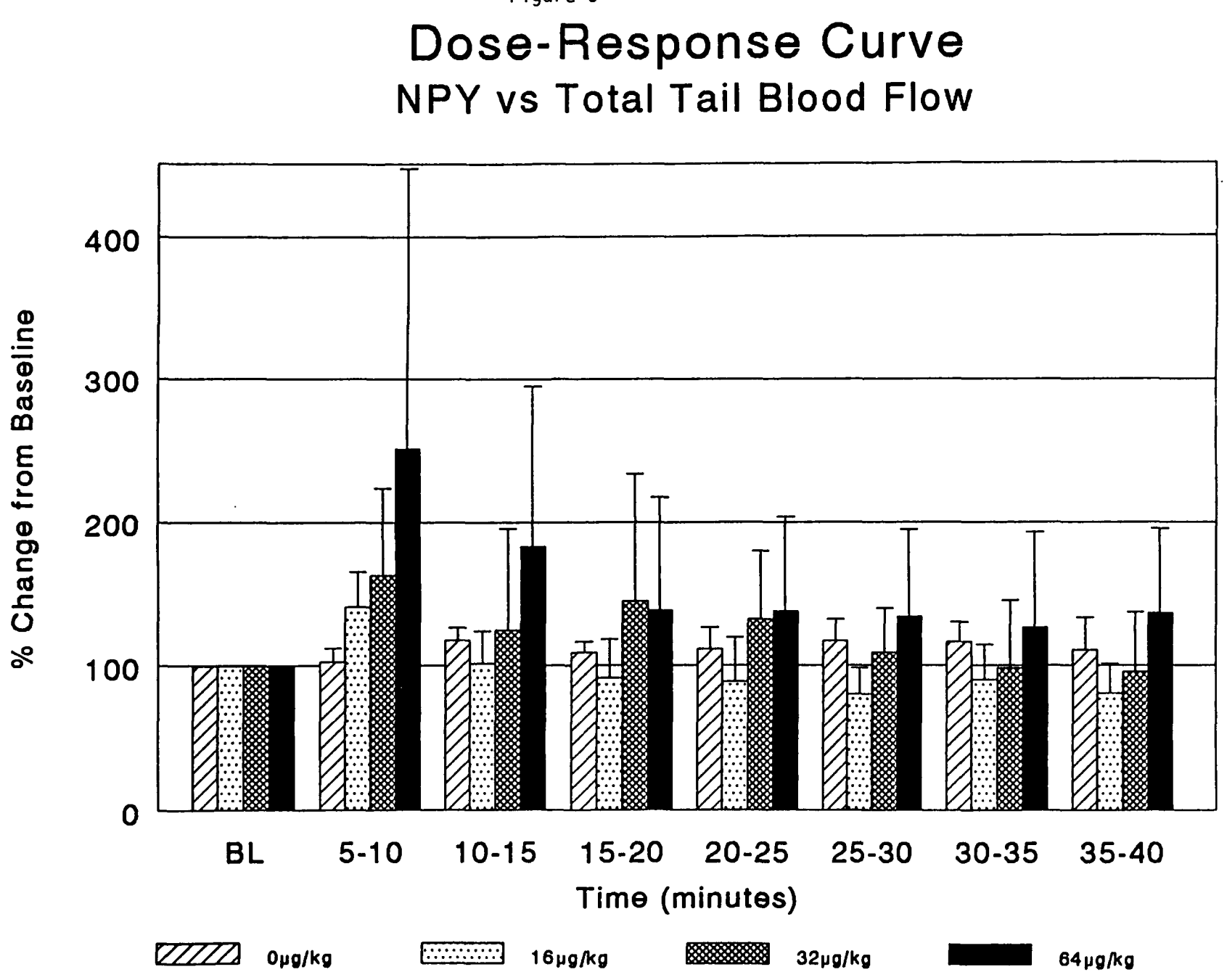


Figure 2b

Figure 3


Dose-Response Curve
NPY vs Total Tail Blood Flow
% Change from Baseline
400
300
200
100
0
BL
5-10
10-15
15-20
20-25
25-30
30-35
35-40
Time (minutes)
0µg/kg
16µg/kg
32µg/kg
64µg/kg

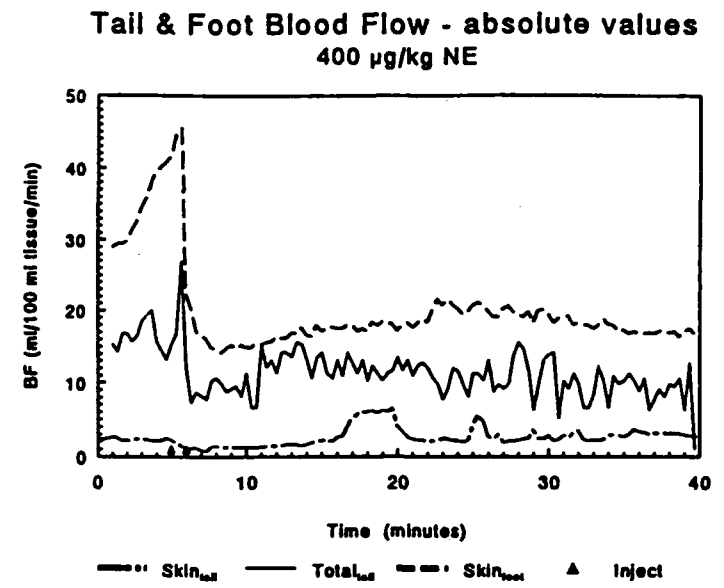


Figure 4a

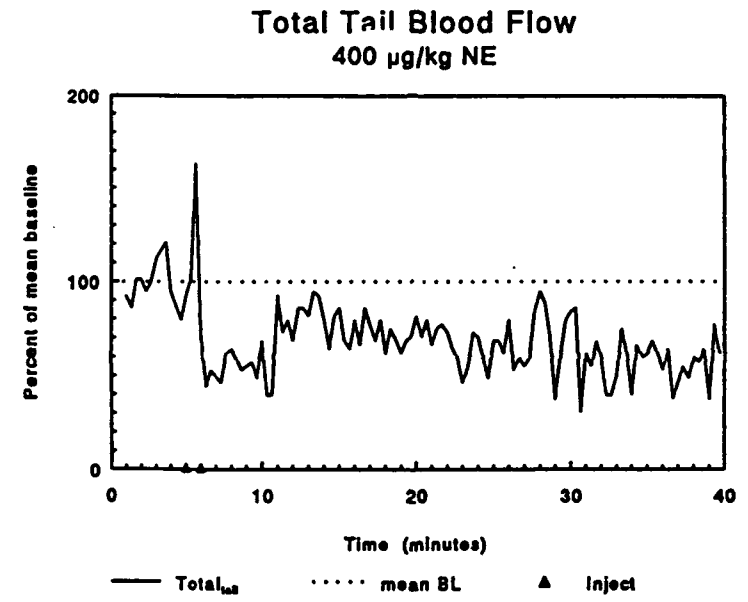


Figure 4b

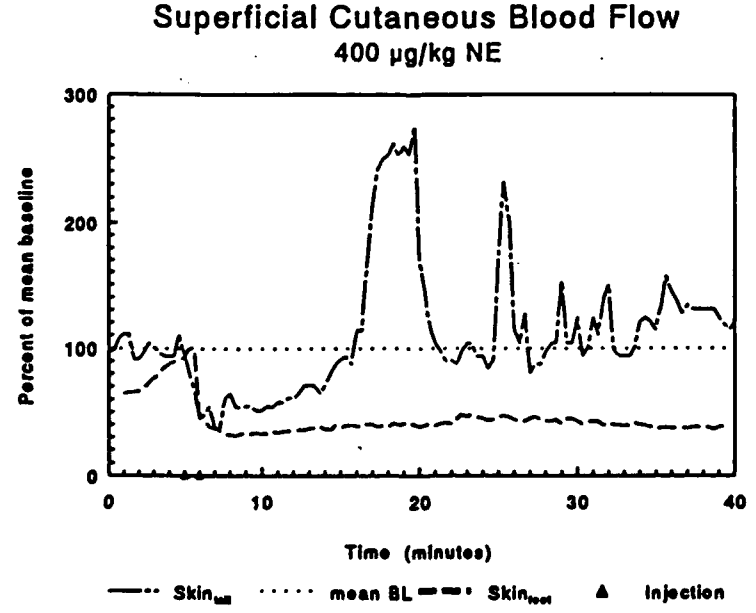


Figure 4c